ICCVAM/NICEATM/ECVAM Scientific Workshop on Alternative Methods to Refine, Reduce, and Replace the Mouse LD_{50} Assay For Botulinum Toxin Testing (November 13-14, 2006)

Panel Discussion Questions

Session 2 Panel Questions: Current Understanding and Knowledge Gaps for Botulinum Toxin[1] (Moderators: James Keller and Ram Ramabhadran)

- What knowledge gaps in the current understanding of the mechanism of action of botulinum toxin must be addressed to develop non-animal replacement methods for potency testing or detection of botulinum toxin?
- To what extent does current research address these knowledge gaps? Does additional effort need to be applied to these areas?
- What research initiatives are necessary to address these knowledge gaps and further characterize mechanisms and modes of action in order to advance the development of non-animal replacement methods for potency testing or detection of botulinum toxin?

Session 3A Panel Questions: Potential Replacement of Animal Use for Botulinum Toxin Potency Testing - Endopeptidase Assays (Moderators: Susan Maslanka and Shashi Sharma)

- Recognizing that it will be necessary to establish that alternative methods are appropriate for each particular pharmaceutical product, can any of the current endopeptidase methods be used now to **replace** animals for potency testing of botulinum toxin? If no, what limiting factors prevent these methods from being used as a replacement for the mouse LD_{50} assay?
- Based on the needs for detecting botulinum toxin in environmental or biological samples (e.g., speed, portability, throughput) as discussed in Session 1, could the endopeptidase assays discussed be used to **replace** animals for these kinds of samples? If no, what limiting factors prevent these methods from being used as a replacement for the mouse LD_{50} assay?
- Can any of the current endopeptidase methods be used now to **reduce** the number of animals used for potency testing of botulinum toxin? If no, what limiting factors prevent these methods from being used to reduce the number of animals used in the mouse LD_{50} assay?
- Based on the needs for detecting botulinum toxin in environmental or biological samples (e.g., speed, portability, throughput) as discussed in Session 1, could the endopeptidase assays discussed be used to **reduce** the number of animals used for these kinds of samples? If no, what limiting factors prevent these methods from being used to reduce the number of animals used in the mouse LD_{50} assay?

[1] There is no panel discussion in Session 1, so there are no panel discussion questions for Session 1.

- Should endopeptidase methods other than those discussed so far during this workshop be considered for development and validation for potency testing or detection of botulinum toxin?
- What are the pros and cons of the different endopeptidase methods reviewed?
- What current knowledge gaps with regard to the reviewed endopeptidase methods must be addressed to further their use in potency testing or detection (as discussed in Session 1) of botulinum toxin? What additional studies are needed?
- Of the endopeptidase methods discussed, which should have the highest priority for further development and validation studies?
- What are the essential characteristics of an endopeptidase method sufficient to replace or reduce the number of animals used for potency testing or detection (as discussed in Session 1) of botulinum toxin?
- What is the best way to assess the validation status of these endopeptidase methods?

Session 3B Panel Questions: Potential Replacement of Animal Use for Botulinum Toxin Potency Testing - Cell-Based Assays (Moderators: Susan Maslanka and Shashi Sharma)

- Recognizing that it will be necessary to establish that alternative methods are appropriate for each particular pharmaceutical product, can any of the current cell-based methods be used now to **replace** animals for potency testing of botulinum toxin? If no, what limiting factors prevent these methods from being used as a replacement for the mouse LD_{50} assay?
- Based on the needs for detecting botulinum toxin in environmental or biological samples (e.g., speed, portability, throughput) as discussed in Session 1, could the cell-based assays discussed be used to **replace** animals for these kinds of samples? If no, what limiting factors prevent these methods from being used as a replacement for the mouse LD_{50} assay?
- Can any of the current cell-based methods be used now to **reduce** the number of animals used for potency testing of botulinum toxin? If no, what limiting factors prevent these methods from being used to reduce the number of animals used in the mouse LD_{50} assay?
- Based on the needs for detecting botulinum toxin in environmental or biological samples (e.g., speed, portability, throughput) as discussed in Session 1, could the cell-based assays discussed be used to **reduce** the number of animals used for these kinds of samples? If no, what limiting factors prevent these methods from being used to reduce the number of animals used in the mouse LD_{50} assay?
- Should cell-based methods other than those discussed so far during this workshop be considered for development and validation for potency testing or detection of botulinum toxin?
- What are the pros and cons of the different cell-based methods reviewed?

- What current knowledge gaps with regard to the reviewed cell-based methods must be addressed to further their use in potency testing or detection (as discussed in Session 1) of botulinum toxin? What additional studies are needed?
- Of the cell-based methods discussed, which should have the highest priority for further development and validation studies?
- What are the essential characteristics of a cell-based method sufficient to replace or reduce the number of animals used for potency testing or detection (as discussed in Session 1) of botulinum toxin?
- What is the best way to assess the validation status of these cell-based methods?

Session 4A Panel Questions: Refinement: Using *Ex Vivo* Assays to Avoid Pain and Distress in Botulinum Testing (Moderators: Elizabeth Shores and Leonard Smith)

- Recognizing that it will be necessary to establish that alternative methods are appropriate for each particular pharmaceutical product, can any of the current *ex vivo* methods be used now to **replace** animals for potency testing of botulinum toxin? If no, what limiting factors prevent these methods from being used as a replacement for the mouse LD_{50} assay?
- Based on the needs for detecting botulinum toxin in environmental or biological samples (e.g., speed, portability, throughput) as discussed in Session 1, could the *ex vivo* assays discussed be used to **replace** animals for these kinds of samples? If no, what limiting factors prevent these methods from being used as a replacement for the mouse LD_{50} assay?
- Can any of the current *ex vivo* methods be used now to **reduce** the number of animals used for potency testing of botulinum toxin? If no, what limiting factors prevent these methods from being used to reduce the number of animals used in the mouse LD_{50} assay?
- Based on the needs for detecting botulinum toxin in environmental or biological samples (e.g., speed, portability, throughput) as discussed in Session 1, could the *ex vivo* assays discussed be used to **reduce** the number of animals used for these kinds of samples? If no, what limiting factors prevent these methods from being used to reduce the number of animals used in the mouse LD_{50} assay?
- Should *ex vivo* methods other than those discussed so far during this workshop be considered for development and validation for potency testing or detection of botulinum toxin?
- What are the pros and cons of the different *ex vivo* methods reviewed?
- What current knowledge gaps with regard to the reviewed *ex vivo* methods must be addressed to further their use in potency testing or detection (as discussed in Session 1) of botulinum toxin? What additional studies are needed?
- Of the *ex vivo* methods discussed, which should have the highest priority for further development and validation studies?
- What is the best way to assess the validation status of these *ex vivo* methods?

Session 4B Panel Questions: Refinement: Alternative *In Vivo* Botulinum Assays that Do Not Require Death as an Endpoint (Moderators: Leonard Smith and William Stokes)

- Recognizing that it will be necessary to establish that alternative methods are appropriate for each particular pharmaceutical product, can any of the current non-lethal *in vivo* methods be used now to **replace** animals for potency testing of botulinum toxin? If no, what limiting factors prevent these methods from being used as a replacement for the mouse LD_{50} assay?
- Based on the needs for detecting botulinum toxin in environmental or biological samples (e.g., speed, portability, throughput) as discussed in Session 1, could the non-lethal *in vivo* assays discussed be used to **replace** animals for these kinds of samples? If no, what limiting factors prevent these methods from being used as a replacement for the mouse LD_{50} assay?
- Can any of the current non-lethal *in vivo* methods be used now to **reduce** the number of animals used for potency testing of botulinum toxin? If no, what limiting factors prevent these methods from being used to reduce the number of animals used in the mouse LD_{50} assay?
- Based on the needs for detecting botulinum toxin in environmental or biological samples (e.g., speed, portability, throughput) as discussed in Session 1, could the non-lethal *in vivo* assays discussed be used to **reduce** the number of animals used for these kinds of samples? If no, what limiting factors prevent these methods from being used to reduce the number of animals used in the mouse LD_{50} assay?
- Should non-lethal *in vivo* methods other than those discussed so far during this workshop be considered for development and validation for potency testing or detection of botulinum toxin?
- What are the pros and cons of the different non-lethal *in vivo* methods reviewed?
- What current knowledge gaps with regard to the reviewed non-lethal *in vivo* methods must be addressed to further their use in potency testing or detection (as discussed in Session 1) of botulinum toxin? What additional studies are needed?
- Of the non-lethal *in vivo* methods discussed, which should have the highest priority for further development and validation studies?
- What is the best way to assess the validation status of these non-lethal *in vivo* methods?

Session 4C Panel Questions: Refinement: Potential Use of Non-Lethal Endpoints in Botulinum LD_{50} Testing to Minimize Pain and Distress (Moderators: William Stokes and Leonard Smith)

- Is there sufficient data to support the use of moribund condition instead of death as an endpoint for the mouse LD_{50} assay? Can this change be implemented now? If not, what studies would be needed to evaluate this alternative endpoint?
- Based on what is known about the progression of botulism in mice, are any other clinical signs sufficiently predictive of mouse lethality that they should be used, or further investigated, as earlier humane endpoints in order to allow for humane euthanasia of mice used in LD_{50} botulinum testing once they are observed?

- Are there objective endpoints (e.g., temperature, heart rate, blood pressure, pO_2) that are sufficiently predictive of mouse lethality that they can be used, or should be further investigated, as humane endpoints to terminate early a mouse LD_{50} test once observed?
- What current knowledge gaps regarding predictive humane endpoints should be addressed in research, development, and validation studies? What additional studies are needed?
- Are there additional data recommended for collection during future animal studies that might aid in identifying and validating more humane, non-lethal endpoints for botulinum toxin testing?

Session 5 Panel Questions: Reduction of Animal Use for *In Vivo* Botulinum Testing (Moderators: Marlies Halder and Richard McFarland)

- Is it feasible to use the mouse LD_{50} assay to assess the potency of batch production samples of botulinum toxin and use a validated *in vitro* and/or *ex vivo* test method to assess potencies of final production lots? Why, or why not?
- Are there validated test method modifications (e.g., use of reference standards) that could be made to the current mouse LD_{50} test method protocol to decrease the number of mice tested?
- Should a reference standard always be used in a validation study conducted on the *in vitro* and *ex vivo* methods discussed at this workshop? Why, or why not? Should an international botulinum toxin reference standard be created for this purpose? If yes, how should it be maintained?
- What are the best practices for minimizing the number of animals used?

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